Rapid Communication

Acute and Subchronic Inhalation Toxicity of Chloroform in Rats and Mice

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Abstract: Acute and Subchronic Inhalation Toxicity of Chloroform in Rats and Mice: Tatsuya Kasai, et al. Japan Bioassay Research Center—In order to better characterize acute and subchronic toxicities of chloroform and to provide its basic toxicity data for risk assessment of humans exposed to chloroform in work and living environments, mice and rats of both sexes were exposed by inhalation to chloroform at different concentrations from 500 to 8,000 ppm for 6 h/d × 5 d/wk × 2 wk and from 12 to 400 ppm for 6 h/d × 5 d/wk × 13 wk. The kidneys, liver and nasal cavity were primarily damaged by the inhalation exposure. Acute death occurred at 12 ppm in male mice, 1,000 ppm in female mice and 2,000 ppm in male and female rats. The 13-wk exposures induced renal lesions in male mice, hepatic and nasal lesions in female mice and renal, hepatic and nasal lesions in male and female rats. Susceptibility to the acute and subchronic toxicities was higher in male mice than female mice, and higher in mice than in rats. No-observed-adverse-effect-levels (NOAELs) and lowest-observed-adverse-effect-levels (LOAELs) were determined for the renal, hepatic and nasal endpoints of animals exposed to chloroform for 13 wk. For the hepatic endpoint, NOAEL was 100 ppm in male mice, 50 ppm in female mice and female rats and 100 ppm in male rats. For the renal endpoint, LOAEL was 12 ppm in male mice, and NOAEL was 25 ppm in male rats and 100 ppm in female rats. For the nasal endpoint, LOAEL was 12 ppm and 25 ppm in the mice and the rats of both sexes, respectively. (J Occup Health 2002; 44: 193–202)

Key words: Chloroform, Rat, Mouse, NOAEL, LOAEL, Liver, Kidney, Nasal organ, Occupational exposure limit

Chloroform has been widely used as an organic solvent, an intermediate for the synthesis of fluorocarbons and drugs, and an insecticidal fumigant. Annual production and importation of chloroform in Japan were reported to amount to 37,000 and 61,000 tons in 2000, respectively¹. Approximately 96,000 workers in the U.S. were exposed to chloroform according to the 1981–1983 National Occupational Exposure Survey². Chloroform is also a major environmental contaminant formed in the chlorination of drinking water in the community and cooling water in power plant and in the process of bleaching paper³. This compound poses a potential hazard to the health of workers and community residents exposed to chloroform in the workplace and in drinking water. The American Conference of Governmental Industrial Hygienists (ACGIH)⁴,⁵ and the Japan Society for Occupational Health (JSOH)⁶,⁷ recommended an occupational exposure limit (OEL) of 10 ppm for chloroform, and a MAK value of 0.5 ppm (2.5 mg/m³) was established in Germany⁸. Those three OELs were derived primarily from bioassay data of rodents exposed to chloroform vapor by inhalation. Acute and chronic toxicities of chloroform in humans and experimental animals have been documented⁹ but there are few published data available for health risk assessment of workers exposed to chloroform through the inhalation route. Inhalation and systemic exposure to chloroform are principal routes for workers, because chloroform is very volatile and permeable through the skin¹⁰. The present study was intended to better characterize acute and subchronic toxicities of chloroform and to provide its basic toxicity data such as no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) for risk assessment of humans exposed to chloroform in work and living environments. We conducted experiments on inhalation exposure of rats and mice of both sexes to chloroform vapor for 2 and 13
Exposure to chloroform pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan). Animals had free access to sterilized water and commercial automatically to give a 12-h light/dark cycle. All the changes per hour. Fluorescent lighting was controlled were described in detail in the previous paper13).

Materials and Methods

Animals

F344/DuCrj rats and Crj:BDF1 mice of both sexes were purchased from Charles River Japan Inc. (Kanagawa, Japan) at 4 wk of age. The animals were quarantined and acclimated for 2 wk before the start of experiment. The animals were housed in six stainless-steel exposure chambers which were installed in a barrier system animal room maintained at a temperature of 22 ± 2°C and a relative humidity of 60 ± 10% with 15–17 air changes per hour. Then the animals were housed individually in stainless-steel wire hanging cages in the stainless steel inhalation exposure chambers maintained at a temperature of 23 ± 2°C and a relative humidity of 55 ± 10% with 12 air changes per hour. Fluorescent lighting was controlled automatically to give a 12-h light/dark cycle. All the animals had free access to sterilized water and commercial pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan).

Exposure to chloroform

The technique for generating chloroform vapor-air mixture and the exposure system used in the present study were described in detail in the previous paper13). Chloroform of reagent grade (purity > 99%) was obtained from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). Each lot of chloroform was analyzed for its chemical purity and stability by gas chromatography and infrared spectrometry, but no impurities or decomposition products were observed.

Experimental design

For the 2-wk exposure study, groups of 10 rats and 10 mice of both sexes were exposed to air containing chloroform vapor at a concentration of 500, 1,000, 2,000, 4,000 or 8,000 ppm for 6 h/d × 5 d/wk × 2 wk. For the 13-wk exposure study, groups of 10 mice of both sexes were exposed to 12, 25, 50, 100 or 200 ppm chloroform vapor, and groups of 10 rats of both sexes to 25, 50, 100, 200 or 400 ppm chloroform vapor for 6 h/d × 5 d/wk × 13 wk. Groups of 10 rats and 10 mice of both sexes were exposed to clean air for 2 or 13 wk under the same conditions, and served as the respective controls.

Clinical observations and analysis, and pathological examinations

All animals were weighed and examined for clinical

Mortality

Table 1 shows mortality rates for rats and mice of both sexes exposed to chloroform vapor for 2 or 13 wk. In the 2-wk exposure study, all the male mice except two died after the first or second day exposure to 500 ppm and above, and the two survived to the end of the 2-wk exposure. All the 500 ppm-exposed female mice survived, and one 1,000 ppm-exposed female mouse survived after the 2-wk exposures. All the male and female rats exposed to 2,000 ppm and above died after the first or second day of exposure.

In the 13-wk exposure study, almost all the exposed male mice died after the first day of exposure, and the other male mice survived throughout the exposures. All the female mice and all the male and female rats survived after the 13-wk exposures to 12 ppm and above and 25 ppm and above, respectively. It was also worth noting that susceptibility to the acute and subchronic toxicities was much higher in male mice than in female mice, and also higher in mice than in rats, but no gender difference between male and female rats was observed in
The chloroform-induced deaths of mice were histopathologically attributed to necrosis (Fig. 1) of proximal tubules in males and to centrilobular necrosis (Fig. 2) of the liver in females. Both male and female rats exhibited no remarkable lesions indicative of the cause of chloroform-induced deaths, but congestion and inflammation of the lungs resulting presumably from cardiovascular effects of chloroform were noted, in addition to the below-mentioned histopathological changes in the kidneys and liver as seen in the rats which survived.

**Table 1. Mortality rates for rats and mice of both sexes exposed to chloroform for 2 or 13 wk by inhalation**

<table>
<thead>
<tr>
<th>Exposed conc.</th>
<th>Mice</th>
<th></th>
<th>Rats</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>2W</td>
<td>0 ppm</td>
<td>0</td>
<td>0</td>
<td>0 ppm</td>
</tr>
<tr>
<td>500 ppm</td>
<td>9 (9/2nd)</td>
<td>0</td>
<td>500 ppm</td>
<td>0</td>
</tr>
<tr>
<td>1,000 ppm</td>
<td>9 (9/2nd)</td>
<td>9 (4/4th) (4/5th) (1/6th)</td>
<td>1,000 ppm</td>
<td>0</td>
</tr>
<tr>
<td>2,000 ppm</td>
<td>10 (10/2nd)</td>
<td>10 (6/2nd) (2/4th) (2/5th)</td>
<td>2,000 ppm</td>
<td>10 (9/1st) (1/2nd)</td>
</tr>
<tr>
<td>4,000 ppm</td>
<td>10 (1/1st) (9/2nd)</td>
<td>10 (10/2nd)</td>
<td>4,000 ppm</td>
<td>10 (9/1st) (1/2nd)</td>
</tr>
<tr>
<td>8,000 ppm</td>
<td>10 (10/1st)</td>
<td>10 (10/1st)</td>
<td>8,000 ppm</td>
<td>10 (10/1st)</td>
</tr>
<tr>
<td>13W</td>
<td>0 ppm</td>
<td>0</td>
<td>0</td>
<td>0 ppm</td>
</tr>
<tr>
<td>12 ppm</td>
<td>2 (1/1st) (1/3rd)</td>
<td>0</td>
<td>25 ppm</td>
<td>0</td>
</tr>
<tr>
<td>25 ppm</td>
<td>9 (8/1st) (1/3rd)</td>
<td>0</td>
<td>50 ppm</td>
<td>0</td>
</tr>
<tr>
<td>50 ppm</td>
<td>10 (7/1st) (3/2nd)</td>
<td>0</td>
<td>100 ppm</td>
<td>0</td>
</tr>
<tr>
<td>100 ppm</td>
<td>8 (8/1st)</td>
<td>0</td>
<td>200 ppm</td>
<td>0</td>
</tr>
<tr>
<td>200 ppm</td>
<td>10 (10/1st)</td>
<td>0</td>
<td>400 ppm</td>
<td>0</td>
</tr>
</tbody>
</table>

The fraction within parenthesis indicates the number of dead animals as the numerator/the day of repeated exposure at death as the denominator.

**Fig. 1.** Necrosis of proximal tubules in the kidney of a male mouse which died after the first day of exposure to 25 ppm chloroform.

**Fig. 2.** Centrilobular necrosis in the liver of a female mouse which died after the second day of exposure to 2,000 ppm chloroform.

Toxicity in surviving animals due to 2-wk exposure

Surviving male mice exposed to 500 and 1,000 ppm exhibited necrosis and cytoplasmic basophilia of proximal tubules in the kidneys, slight swelling and vacuolar change in the liver, and atrophy and respiratory metaplasia in the olfactory epithelium. The female mice exposed to 500 and 1,000 ppm exhibited necrosis and vacuolar change in the central area of the liver, and degeneration, necrosis and disarrangement of the olfactory and respiratory epithelium, but no kidney lesions were observed in the exposed female mice. Surviving male and female rats exposed to 500 and 1,000 ppm had vacuolar changes in proximal tubules of the kidneys and in the central area of the liver, in addition to desquamation, atrophy and disarrangement of the olfactory epithelium and edema of the lamina propria of the nasal cavity.

Subchronic toxicity due to 13-wk exposure

**General observations:** No clinical signs or exposure-related changes in gross findings were observed in either rats or mice exposed to chloroform for 13 wk. A
Histopathological changes

Incidences of the main lesions in the animals exposed to chloroform vapor for 13 wk are summarized in Table 2.

Mice: A statistically significant increase in the incidence of necrosis of proximal tubules was found in the males exposed to 12 ppm and above, whereas the tubular degeneration was found at 12 ppm but its incidence significantly increased at 25 ppm and above. Cytoplasmic basophilia (Fig. 3) of proximal tubules was observed in all the male mice which survived after the 13 wk exposure to chloroform, and its incidence was statistically significant at an exposure level of 12 ppm. On the other hand, cytoplasmic basophilia was not found in the male mice which died acutely after the first or second-day exposure to chloroform. Cytoplasmic basophilia was found only in a female mouse exposed to 100 ppm. The exposed females exhibited different histopathological changes in the liver and their incidences from the exposed males (Table 2). Necrosis and cell atypia of the liver (Fig. 4) were found in all the female mice exposed to 200 ppm and 100 ppm and above, respectively. Liver swelling did not occur in any exposed female, whereas this lesion was observed in all the males exposed to 200 ppm. There was also a gender difference in nasal lesions in the exposed mice (Table 2). A significantly increased incidence of degeneration of the olfactory epithelium was observed in the males exposed to 25 ppm and above, but that lesion did not occur in any exposed females. Statistically significant increases in

Urinary and biochemical parameters

The urinary and blood biochemical parameters of both rats and mice of both sexes after inhalation exposure to chloroform for 13 wk are shown in Table 3. No significant difference between the 12 ppm-exposed male mice and the respective control was found in any blood biochemical parameters. Urinary protein was found in the male mice exposed to 12 ppm. Significant increases in serum levels of GOT, GPT and ALP were noted in the females exposed to 200 ppm and above, whereas those two lesions in male rats appeared at higher exposure levels than in females. Significant increases in serum levels of glucose were observed only in male and female rats exposed to 200 ppm and above. Occult blood was observed only in the 25 ppm-exposed male mice. Serum levels of ALP, LAP and γ-GTP significantly increased in the male rats exposed to 200 and 400 ppm and in the male rats exposed to 400 ppm, as compared to those observed in the respective controls. Positive urinary glucose was observed only in male and female rats exposed to 400 ppm, whereas the serum level of glucose significantly decreased in the male rats exposed to 50 ppm and above and in the female rats exposed to 200 and 400 ppm. Occult blood occurred in the male rats exposed to 50 ppm and above and in the female rats exposed to 200 and 400 ppm. Urinary protein was found in the male rats exposed to 400 ppm.
### Table 2. Incidences of principal lesions in mice and rats of both sexes in the 13-wk exposure study

#### (A) Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>12 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Number of animals used</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

**Nasal cavity**

- Degeneration of olfactory epithelium: 
  - Male: 0 (2/2), 5** (5/9), 7** (7/10), 6** (6/8), 9** (9/10)
  - Female: 0, 0, 0, 0, 0, 0, 0, 0, 0

- Thickening of bone: 
  - Male: 0, 7** (7/10), 0, 0, 0, 7** (7/10), 9** (9/10)
  - Female: 0, 0, 0, 0, 0, 0, 0

- Eosinophilic change of olfactory epithelium: 
  - Male: 0, 0, 0, 1, 0, 0, 0, 8** (8/10), 10** (10/10)
  - Female: 0, 0, 0, 0, 0, 0, 0

- Eosinophilic change of respiratory epithelium: 
  - Male: 0, 0, 0, 1, 0, 0, 0, 8** (8/10), 7** (7/10)
  - Female: 0, 0, 0, 0, 0, 0, 0

**Liver (central)**

- Swelling: 
  - Male: 0, 0, 0, 0, 0, 10** (10/10)
  - Female: 0, 0, 0, 0, 0, 0

- Cell atypia: 
  - Male: 0, 0, 0, 0, 0, 0, 0, 0, 0
  - Female: 0, 0, 0, 0, 0, 0, 0, 0, 0

- Necrosis: 
  - Male: 0, 0, 0, 0, 0, 0, 0, 0, 0
  - Female: 0, 0, 0, 0, 0, 0, 0, 0, 0

- Vacuolic change: 
  - Male: 0, 0, 0, 0, 0, 0, 0, 0, 0
  - Female: 0, 0, 0, 0, 0, 0, 0

**Kidneys (proximal tubules)**

- Tubular Necrosis: 
  - Male: 0, 7** (7/9), 10** (10/10), 9** (9/10), 10** (10/10)
  - Female: 0, 0, 0, 0, 0, 0

- Degeneration: 
  - Male: 0, 2, 9** (9/10), 10** (10/10), 8** (8/10), 10** (10/10)
  - Female: 0, 0, 0, 0, 0, 0

- Cytoplasmic basophilia: 
  - Male: 0, 8** (8/10), 1, 0, 2, 0
  - Female: 0, 0, 0, 0, 0, 0

The parenthesis indicates the number of dead animals bearing a lesion/total number of animals which died during the 13-wk exposure period.

#### (B) Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>25 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Number of animals used</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

**Nasal cavity**

- Mineralization: 
  - Male: 0, 10** (10/10), 10** (10/10), 10** (10/10), 10** (10/10)
  - Female: 0, 10** (10/10), 10** (10/10), 10** (10/10), 10** (10/10)

- Atrophy of olfactory epithelium: 
  - Male: 0, 7** (7/9), 10** (10/10), 9** (9/10), 10** (10/10)
  - Female: 0, 0, 0, 0, 0

- Necrosis of olfactory epithelium: 
  - Male: 0, 0, 0, 8** (8/10), 10** (10/10)
  - Female: 0, 0, 0, 0, 0

**Liver (central)**

- Deposit of ceroid: 
  - Male: 0, 0, 0, 0, 0, 0, 0, 1
  - Female: 0, 0, 0, 0, 0, 0, 0, 1

- Collapse: 
  - Male: 0, 0, 0, 10** (10/10), 10** (10/10), 0, 0, 8** (8/10), 9** (9/10)
  - Female: 0, 0, 0, 0, 0, 0, 0, 0

- Vacuolic change: 
  - Male: 0, 0, 0, 0, 0, 2, 0
  - Female: 0, 0, 0, 0, 0, 2

**Kidneys (proximal tubules)**

- Vacuolic change: 
  - Male: 0, 0, 0, 3, 4* (4/9), 0, 0, 2, 6** (6/10)
  - Female: 0, 0, 0, 0, 0, 2, 6** (6/10)

Significant difference; *: P≤0.05, **: P≤0.01 by Chi-square test. The animals which died during the exposure period are included in statistical analysis.
Fig. 3. Cytoplasmic basophilia in the kidney of a surviving male mouse exposed to 12 ppm chloroform for 13 wk.

Fig. 4. Necrosis and cell atypia in the central area of the liver of a female mouse exposed to 200 ppm chloroform for 13 wk.

Fig. 5. Thickening of bone in the septum of the nasal cavity of a female mouse exposed to 200 ppm chloroform for 13 wk.

Fig. 6. Vacuolic change of the epithelium of proximal tubules in the kidney of a female rat exposed to 400 ppm chloroform for 13 wk.

Fig. 7. Collapse, e.g., the loss of hepatocytes, and ceroid deposit in the central area of the liver of a male rat exposed to 400 ppm chloroform for 13 wk.

Fig. 8. Necrosis and atrophy of the olfactory epithelium and mineralization in the ethmoturbinate of the nasal cavity of a male rat exposed to 200 ppm chloroform for 13 wk.
Chloroform toxicity in rodents

It has been recognized that chloroform is metabolized primarily through the oxidative pathway to phosgene by the cytochrome P450, and that phosgene but not chloroform itself is responsible for manifestation of toxicity of inhaled or ingested chloroform. It was also reported that the CYP2E1 isozyme is enriched in the kidneys, liver and nasal organ. It was found in the present study that the kidneys, liver and nasal cavity are the principal organs damaged by acute and subchronic inhalation exposure to chloroform. Lesions in these three organs may be caused by either enhanced deposition of or site-specific tissue susceptibility to chloroform and/or its metabolite. Since tissue levels of inhaled chloroform were reported to be distributed widely throughout the body, it can be inferred that the acute and subchronic toxicities of inhaled chloroform are brought about by phosgene, depending on the localized metabolic capacity of the P450 enriched tissues such as the kidneys, liver, and nasal organ.

In the present study, it was found that mice are more susceptible to acute and subchronic toxicities of inhaled chloroform than rats, as evidenced by a much lower lethal concentration in male mice than in female mice (Table 1) as well as lower LOAEL (12 ppm) for proximal tubular necrosis in male mice than in female mice (no tubular necrosis detected in any sex).
The gender difference in susceptibility to chloroform toxicity between male and female mice is consistent with the result reported by Smith et al., and can be explained in terms of greater metabolic capacity of renal CYP2E1 isozyme activities in males than in females. No gender difference in susceptibility between male and female rats found in the present study can be accounted for by a modest difference in the CYP2E1 activity in male and female rat kidneys.

Kidney, liver and nasal lesions

The kidney lesions in male mice were characterized by necrosis, degeneration and cytoplasmic basophilia of proximal tubules occurring at the lowest exposure level of 12 ppm. No such kidney lesions were found in the exposed female mice, but the significantly increased BUN indicative of functional failure of the kidneys was observed in the female mice exposed to 50 ppm and above. The cytoplasmic basophilia which was observed in all 8 surviving male mice exposed to 12 ppm, indicative of the regenerative and proliferative response to necrosis, is consistent with the result reported by Larson et al. who observed increased cell proliferation as indicated by bromodeoxyuridine incorporated into the kidneys of male B6C3F1 mice exposed to 10 ppm or below for 13 wk. The rat kidney lesions were characteristic of vacuolic change (fatty change) which might result from the necrosis, occurring at high exposure levels of 200 and 400 ppm. The present findings in the occult blood of the male and female rats, the positive urinary protein in the male rats and male mice and the increased BUN in the female mice may reflect functional failure of the kidneys. The positive urinary glucose occurring in the 400 ppm-exposed rats of both sexes may correspond to the marked decrease in the blood glucose in the same 400 ppm-exposed groups and be causally related to malfunctioning glucose reabsorption in proximal tubules. The present results on renal vacuole change observed in the rats of both sexes exposed to the high exposure levels are consistent with those reported by Templin et al. who reported that vacuolation of proximal tubules was observed only at 30 ppm for male and at 90 ppm for female mice.

It was striking that in the present study the olfactory region of the nasal cavity of rats and mice of both sexes was very susceptible to chloroform exposure, because the nasal lesions occurred at the lowest exposure level of 12 ppm in mice and 25 ppm in rats. Since cytochrome P-450 is reported to be enriched in the olfactory region of the nasal cavity, the chloroform-induced lesions in the nasal cavity may be explained plausibly in terms of phosgene, a reactive metabolite of chloroform, rather than a direct action of the inhaled chloroform on the nasal tissues. The severity and spatial patterns of the nasal lesions in the chloroform-exposed rats and mice found in the present study are in essential agreement with those of bromodeoxyuridine immunolabeling following exposure of rats and mice to 300 ppm chloroform reported so far, but Mery et al. suggested that CYP2E1 was not preferentially distributed in the regions in which the chloroform-induced nasal lesions occurred. Further study will be needed to explore other causative factors responsible for the nasal lesions, in addition to phosgene. There was a gender difference in the pattern of the nasal histopathology between male and female mice, because the degeneration of olfactory epithelium was observed in males, whereas the eosinophilic changes of olfactory and respiratory epithelia were observed in females. No such a gender difference was found between male and female rats.

Species difference in toxicity

Intentional or accidental exposure of humans to chloroform was reported to result in increased serum biomarkers of GOT, GPT and LDH indicative of liver cell necrosis followed by increased liver regeneration biomarkers of γ-GTP, alphafetoprotein and retinol-binding protein, symptoms of jaundice and hepatic coma, and liver degeneration and necrosis at autopsy. Occupational exposure to chloroform at about 1,950 ppm was reported to induce jaundice among 5 of 13 workers and to result in 18 cases of jaundice among factory workers who were exposed to chloroform of 80 to 160 ppm for less than 4 months. Kluwe reported that chloroform is readily metabolized in humans, presumably by the liver.
Knowledge about a species difference in the susceptibility to chloroform toxicity between humans and rodents is of prime importance to extrapolate the rodents’ toxicity data to humans. The results of a physiologically based pharmacokinetic (PBPK) model for chloroform\(^{12}\) suggested that the metabolic activation of chloroform to a toxic intermediate, phosgene, occurs most rapidly in mice, less rapidly in rats, and most slowly in humans. Amet et al.\(^{25}\) reported that human kidney microsomes do not contain a significant amount of CYP2E1 and that the microsomal monoxygenase activities were much higher in the human liver than in human kidneys. Taking the above-mentioned findings\(^{23,33}\) into consideration, human acute chloroform poisoning may be characterized by the liver damage rather than kidney damage.

**NOAELs and LOAELs for OEL**

NOAELs and LOAELs for various endpoints of the exposed mice and rats can be determined from dose-response relationships for the endpoints of general health status such as body and organ weights, histopathological changes and blood biochemical and urinary parameters. It was found in the present 13-wk inhalation exposure of rats to chloroform vapor that a value of 25 ppm is the LOAEL for the nasal endpoint and the NOAEL for the suppressed growth rate, increased blood glucose and positive occult blood. In the present mouse study, a value of 12 ppm was the LOAEL for the renal and nasal endpoints of histopathology and for the blood biochemical endpoint of the increased urinary protein and the NOAEL for the increased BUN. The NOAELs and LOAELs determined in the present study are comparable to those reported by Larson et al.\(^{23}\) and Templin et al.\(^{26}\) who found a concentration of 10 ppm to be the experimental NOAEL for induced proliferation in proximal tubules of male and female F344 rats and in the liver of female mice. Interestingly, the LOAELs of 25 ppm and 12 ppm for the nasal endpoint of the exposed rats and mice found in the present study, respectively, are comparable to those reported by Mery et al.\(^{25}\). Because the biological significance of the nasal histopathology of the chloroform-exposed rodents remains unclear, further study will be needed to correlate the histopathological lesions in the nasal organ with possible functional impairments of olfactory sensation, as demonstrated by a positive correlation between functional impairment of olfactory sensation and nasal histopathology of the severe degeneration of the olfactory region in the 3-methylindole-administered rat\(^{36}\).

The existing OEL values for chloroform\(^{24,6,7}\) among ACGIH-TLV, JSOH-OEL and German MAK were derived primarily from the bioassay data of rodents exposed to chloroform vapor by inhalation. The value of 10 ppm set by both ACGIH-TLV and JSOH-OEL was derived from two independent studies which reported that repeated 7-h exposure of male rats to chloroform at 25–30 ppm increased the relative liver and kidney weights\(^{35,36}\), whereas repeated 4-h exposure to the same concentration did not produce any liver lesion\(^{36}\). The 10 ppm value is approximately equivalent to the 4-h exposure of rats to 25 ppm\(^{36}\). On the other hand, the German MAK value of 0.5 ppm was based on a NOAEL of 5 ppm for an endpoint of increased cell proliferation in the liver and kidneys after inhalation exposure of rats and mice to chloroform for 13 wk\(^{8}\). The cytotoxicity and subsequent cell proliferation in the kidneys and liver of the chloroform-exposed rodents are considered to be a crucial step in the development of chloroform-induced tumorigenesis as a non-genotoxic and cytotoxic mode of action\(^{25,26,37,38}\). In our companion paper\(^{12}\), it was reported that two-year inhalation exposure of male mice to chloroform produces the sustained cytoplasmic basophilia in significant association with the renal cell adenoma and carcinoma. Therefore, the LOAELs and the NOAELs determined in the present study would provide additional information about basic toxicity data for risk assessment of workers exposed to chloroform by inhalation.

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